Title: Chronic Granulomatous Disease *GeneReview* – Research Testing

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Research Testing

Determination of residual superoxide function is important in management: patients with little to no superoxide production are at the greatest risk for mortality [Kuhns et al 2010] and, thus, are the most likely candidates for HSCT. The following three tests are performed in research laboratories only:

- Cytochrome *c* reduction assay quantitates indirectly the actual amount of superoxide produced by measuring spectrophotometrically the inhibitable reduction of ferricytochrome *c* by superoxide dismutase to ferrocytochrome *c* [Elloumi & Holland 2014]. Results of this test correlate well with results of the DHR test.
- Chemiluminescence. Because superoxide can cause a variety of chemical agents to luminesce, measurement of luminescence (typically using dichlorofluoroscein [DCF]) can quantitate the amount of superoxide produced [Elloumi & Holland 2014]. While this assay can rapidly detect superoxide activity and identify hypomorphic forms of CGD, it lacks cellular resolution and thus cannot identify female carriers of X-linked CGD.
- Neutrophil superoxide production of reactive oxygen intermediates (ROI). The quantitation of superoxide produced can be obtained directly from the cytochrome *c* reduction assay (a research laboratory test) or indirectly from the DHR test (a routine clinical test). In general, a DHR test value in the lower range (i.e., <225 arbitrary units) correlates with poor superoxide production, which can be predicted from the specific NADPH oxidase pathogenic variant (see Genotype-Phenotype Correlations).

Immunoblot test for the NADPH complex proteins. Failure to detect the following cytoplasmic subunits of the phagocyte NADPH oxidase (phox) proteins suggests autosomal recessive inheritance: p47^{phox} (encoded by *NCF1*), p67^{phox} (*NCF2*), or p40^{phox} (*NCF4*) (Table 1 and Table A). Immunoblotting is currently performed only in research laboratories.

Note: This technique cannot distinguish between pathogenic variants in *CYBB* (encoding gp91^{phox}) and *CYBA* (encoding p22^{phox}). Because the protein products of these two genes stabilize each other within the phagocyte membrane, absence of one protein results in the absence of the other [Segal et al 2000] (see Molecular Genetics). Pathogenic variants in *CYBB* or *CYBA* that cause a failure to bind heme (leading to a loss of the cytochrome b558) have been referred to as cytochrome negative. In contrast, pathogenic variants in *NCF1*, *NCF2*, and *NCF4* leave cytochrome b558 intact and have been referred to as cytochrome positive. Because pathogenic missense variants in either *CYBB* or *CYBA* can also support cytochrome b558 persistence without function, the terminology ‘cytochrome negative’ and ‘cytochrome positive’ is not preferred.

References

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